CRC Handbook of Endocrinology

Editors

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and

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PREFACE

Endocrine function is reviewed in 17 topic-oriented chapters in this multi-authored handbook, emphasis being placed on the basic science approach. Each chapter includes a selected survey of the existing vast experimental literature.

The handbook was developed as a general reference source for the academic endocrinologist, teacher and researcher, and graduate and undergraduate student. It provides a source of reference for all persons interested in endocrinology and is addressed to a broad audience. The medical aspects of endocrinology, including symptoms, clinical assays, diagnosis and treatment regimens of specific diseases, are available elsewhere in many excellent texts.

Since the ultimate interest of many readers may lie in endocrine pathology, this text is directed toward developing the bases that are needed to understand endocrine action and control. The descriptions thus include anatomic and histologic data as well as physiologic and biochemical studies and interpretations. Since a unitary feature of the endocrine system is the regulation of bodily processes, the control mechanisms are emphasized. The descriptions emphasize the close alliance of the endocrine and nervous systems in homeostasis, particularly the central nature of neuroendocrine control through the hypothalamus via the pituitary to the target cells.

Overall, the reader will have access to a condensed but comprehensive survey of the chemical nature of hormones, their synthesis, secretion and transport, their actions and mechanisms of action, and their degradation and excretion, in mammals including man.

THE EDITORS

Dr. George H. Gass is presently Chairman, Department of Basic Sciences, of the Oklahoma College of Osteopathic Medicine and Surgery in Tulsa, Oklahoma. Previously he held the position of Director of the Endocrinologic Pharmacology Research Laboratory at Southern Illinois University for 18 years, during which time he was also a professor of Physiology and a professor of Medicine. He has had a very diverse career, spanning industry (Lederle Laboratories), government (Food and Drug Administration), and in university and college higher education surroundings.

Dr. Gass was awarded his doctorate at Ohio State University in 1955. His research was on the effects of androgens and their interrelationships with endocrine organs. Following graduation from Ohio State University, Dr. Gass served in the Endocrine Branch of the Food and Drug Administration in Washington, D.C. where he performed biological assay procedures, biostatistics, and endocrine research for four years before leaving to enter higher education. Dr. Gass' best known work in the Food and Drug Administration was in the co-development of the uterine weight method for estrogen assay and detection. Dr. Gass assumed his duties at Southern Illinois University, Department of Physiology, in the fall of 1959 and almost immediately upon arrival set up the Endocrinologic Pharmacology Research Laboratory. A number of students obtained their research experience under Dr. Gass in the Endocrinologic Pharmacology Research Laboratory where it was first discovered that a quantitative measure of chemical carcinogen (diethylstilbestrol)—dose response relationship of mammary tumors existed. This work has become a classic, and although published in 1964, has just recently been repeated by the National Center for Toxicological Research with Dr. Gass as a consultant.

Dr. Gass, while a member of the staff at Southern Illinois University, received a large number of honors and served on numerous occasions as a consultant for government and industry. Dr. Gass is a Fellow of the American Association for the Advancement of Science, an Alexander Van Humbolt Fellow, and a Fulbright alumnus.

He was requested to serve as a consultant for the National Center of Toxicology, Food and Drug Administration, to help determine the carcinogenicity and estrogenecity of female sex hormones, both naturally occurring and synthetic. During his 18 years at Southern Illinois University he taught physiology and pharmacology continuously. His present position as Chairman, Department of Basic Sciences, at the Oklahoma College of Osteopathic Medicine and Surgery allows him to come into intimate contact with the basic scientists in the college including those in the disciplines of human anatomy, histology, pharmacology, physiology, behavior, and biochemistry.

Dr. Harold M. Kaplan has had broad experience in many facets of biologic and medical sciences. His publications, over 200 in number, range through a diverse series of research disciplines, spanning a period of 45 years. He is an author of seven textbooks in anatomy and physiology.

Dr. Kaplan completed his doctorate at Harvard University in 1933, his research then centering on the biochemistry of lipids. After one subsequent year, on the Harvard staff, he entered teaching as a career, working successively in several universities. In his early activities he chaired the Departments of Physiology at the Middlesex Medical School, at the Middlesex Veterinary School, and then at Brandeis University. He taught at the University of Massachusetts, and then went to Southern Illinois University where he chaired the Department of Physiology for 22 years. He is currently Visiting Professor of Physiology at the Southern Illinois University School of Medicine.

Dr. Kaplan has served in a large number of capacities on a national scale, including the presidency of the American Association of Laboratory Animal Science, and also

of the Illinois State Academy of Science. He was chairman of the Editorial Board of Laboratory Animal Science and is currently an associate editor of that journal. He serves on the Board of Directors of the Illinois Society for Medical Research and was on the Board of the Illinois State Academy of Science. He was for several years on the Advisory Council of the Institute of Laboratory Animal Resources. He has taught human physiology continuously since 1935.

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TABLE OF CONTENTS

HYPOTHALAMIC HORMONES AND OTHER FACTORS

Eldon L. Nelson, Jr.

HISTORICAL OVERVIEW

Only since 1955 have we formally entertained the concept that the pituitary, once recognized as the "master gland," is itself precisely controlled by regulatory agents secreted by the hypothalamus.[1,2] These hypothalamic substances controlling pituitary hormone secretion are termed "hypophysiotropic agents." For ten years there was a vigorous search for such substances, but the isolation, purification, and identification of these "hormones" ended without success. In 1965, a major effort was initiated with concurrent studies in the separate laboratories of Schally and Guillemin[3,4,5] to reinforce the then dying hypothesis of hypothalamic hormonal regulators of anterior pituitary function. They reprocessed tons of porcine and ovine brain extracts derived from previous vain attempts that had been performed to isolate corticotropin releasing factor, CRF. Their new, and quite possibly final, search was for a hormone proposed to regulate the pituitary secretion of thyroid stimulating hormone (TSH). By 1969, thyrotropin releasing hormone (TRH) was isolated, purified, and its chemical structure identified as a tripeptide (pyroGlu-His-pro-NH_2). TRH was the first hypophysiotropic hormone proven to be present in the hypothalamus. Within three years, the second hypothalamic hypophysiotropic hormone, luteinizing hormone releasing hormone (LHRH), was isolated, purified and it's decapeptide structure defined. Since then, growth hormone release-inhibitng hormone (somatostatin) has been characterized and significant progress has been made to identify other hypothalamic factors. For their creativity and perseverance, both Schally and Guillemin received the Nobel Prize in 1977. The exciting history of events of their search for the hypothalamic hypophysiotropic agents is documented in a series of articles in *Science*.[3-5]

During the subsequent years, the continuing search to understand the regulatory influence of the hypothalamus has revealed numerous other factors yet to be chemically elucidated (Table 1). It is recognized that these peptidergic agents may have important effects far beyond the regulation of pituitary endocrine secretion.[6-8] Techniques for localization and isolation of the releasing agents and other factors are described in more detail elsewhere.[7-10] Because of numerous technical difficulties, the capability of measuring hypothalamic peptides and ascertaining their location and biosynthesis is severely hindered and the studies reported are influenced by these deficiencies.[10,11] Several reviews of the state of knowledge of hypothalamic peptide biosynthesis offer excellent descriptions of the procedures and difficulties entailed.[7,12,13] It is the intent in this present chapter to present a concise, current and general description of all hypothalamic hypophysiotropic agents known to date. Literature cited here, will refer to selected recent reviews, papers of historical interest, and current papers of importance. The experimental evidence for many observations, procedures, and hypotheses are not thoroughly covered as they are presented in detail by the authors of the referenced papers. The Tables have been presented to provide concise information that would otherwise add laborious reading for those not particularly interested in the detail presented on that topic.

The discussion will first be directed to those hypothalamic hormones and factors that stimulate the release of other hormones from the pituitary, followed by a discussion of hypothalamic agents that inhibit anterior pituitary secretion. A final section will present a general description of most of the other peptide factors identified within the hypothalamus.

NOMENCLATURE

The literature contains a bewildering array of terms describing the hypothalamic regulatory agents. The present chapter employs the terminology recommended by Schally et al.[14] to delineate between factors and hormones. A hypothalamic factor is a substance shown to have regulatory effect upon the pituitary release of hormones, but which has yet to have its chemical stucture identified. A hypothalamic hormone is a chemically characterized agent shown to regulate hormonal secretion from the pituitary. Any impure extract showing "activity" is considered to contain a "factor." The abbreviations utilized for the agents will be the first abbreviations shown for each agent in Table 1.

HYPOPHYSIOTROPIC RELEASE-STIMULATING HORMONES AND FACTORS

Corticotropin Releasing Factor (CRF)

Identification

Although CRF was essentially the first hypophysiotropic substance extracted from the hypothalamus, its structure has yet to be defined. Progress has been hampered by the lack of a simple, definitive, and reliable assay for CRF activity. Some reports suggest CRF is structurally similar to either the neurohypophyseal hormone vasopressin (ADH) or the hypothalamic melanocyte stimulating hormone (MSH).[14] For some time ADH was thought to be the elusive CRF, since ADH is found in the hypothalamus and causes the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. Synthetic analogs of ADH have varying degrees of CRF activity.[15] A review of numerous studies comparing ADH and CRF is found elsewhere.[16] The most convincing argument that they are separate entities is the presence of substantial CRF activity in hypothalamic extracts of rats with inherited deficiency of ADH (Brattleboro strain). Most recent studies of highly purified porcine hypothalamic extracts suggest that CRF is approximately 4,000 daltons and loses its activity in the presence of trypsin or thermolysin, indicating that it is a basic polypeptide.[14]

There may be more than a single hypothalamic factor controlling ACTH release.[16]

Location and Secretion

Bioassayable CRF appears to be most concentrated in the medial basal hypothalamus,[17] though CRF activity is found not only within the hypothalamus, but throughout the brain and activity also occurs in peripheral tissues.[15,18]

The release of CRF is under the influence of central nervous system (CNS) and while there is controversy, CRF secretion usually is enhanced by serotonin (5-HT) and acetylcholine and inhibited by norepinephrine (NE) and gamma aminobutyric acid (GABA).[17] A model proposing the interaction of these agents upon CRF secretion is presented elsewhere.[16] CRF is released in an episodic fashion with a variable frequency that reflects a circadian rhythm in blood levels of ACTH and hydrocortisone. These levels are higher in the morning and lower in the evening hours.

Physiologic Role

CRF is formed within the median eminence (ME) in specific peptidergic neurons and thought to be released into the hypothalamic-hypophyseal portal system. It is then carried to the anterior pituitary where, acting upon specific cells (corticotrophs), it causes the enhanced synthesis and release of ACTH. ACTH is released into the peripheral systemic blood supply, and carried to the adrenal cortex where it acts upon specific cells, increasing the production and release of several adrenal steroids. The levels of the primary human glucocorticoid, hydrocortisone, increases in the peripheral blood

Table 1
HYPOTHALAMIC HORMONES AND FACTORS

Name	Abbreviations	Structure
Releasing Agents		
Corticotropin releasing factor	CRF	Not identified
Thyrotropin releasing hormone	TRH	pGlu-His-Pro-NH_2
Luteinizing releasing hormone	LRH, LHRH, FSHRH, GnRH, LRF	PGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
Somatotropin releasing factor	SRF, GRF, GHRF	Not identified
Prolactin releasing factor	PRF	Not identified
Melanocyte stimulating hormone releasing factor	MRF, MSHRF	H-Cys-Tyr-Ile-Gln-Asn-OH[a]
Release-inhibiting Agents		
Somatostatin	SS, GIF, GHIF	
Prolactin release-inhibiting factor	PIF (Dopamine)[a]	3,4-dihydroxyphenylethylamine[a]
Melanocyte stimulating hormone release inhibiting factor	MIF, MSHIF	H-Pro-Leu-Gly-NH_2[a]
Other Hypothalamic Agents		
Vasopressin	ADH	Glycinamide-Arg-Pro-Cys-Tyr-Phe-Gln-Asn
Ocytocin	MLF	Glycinamide-Leu-Pro-Cys-Tyr-Ile-Gln-Asn
Substance P	SP	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Gly-Leu-Met
Neurophysins		Not identified
Hypothalamic Opiates	leu-Enkephalin	Tyr-Gly-Gly-Phe-Leu-OH
	met-Enkephalin	Tyr-Gly-Gly-Phe-Met-OH
	α-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser HO-Thr-Val-Leu-Pro-Thr-Gln
	β-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser [Asn-Lys-Phe-Leu-Thr-Val-Leu-Pro-Thr-Gln / Ala-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Gly] HO-Gln .
	γ-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser HO-Leu-Thr-Val-Leu-Pro-Thr-Gln
Neurotensin	NT	<Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro HOOC-Leu-Ile-Tyr
Vasoactive intestinal peptide	VIP	His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr [Lys-Val-Ala-Met-Gln-Lys-Arg-Leu-Arg-Thr / Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2]
Angiotensin II	AII	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Cholecystokinin	CCK	Lys-Ala-Gly-Pro-Ser-Arg-Val-Ile-Met [Ser-Pro-Leu-His-Gln-Asn-Asn-Lys-Ser / Arg-Ile-Asp-Ser-Arg-Asp-Tyr-Met] NH_2-Phe-Asp-Met-Trp-Gly

[a] Structure or substance not confirmed.

and returns to the region of the hypothalamus and pituitary where it thwarts the release of, or hampers the pituitary response to, CRF. A classic long negative-feedback regulatory mechanism is thus exemplified.

CRF and perhaps other hypothalamic hormones may possess important physiologic functions other than to act directly upon the adenohypophysis. CRF has an ubiquitous distribution within the Central Nervous System (CNS) and is found associated most often with synaptosomal fractions of brain extract. Electrical stimulation and elevated concentrations of K^+ can increase the release of CRF from isolated nerve endings of the Median Eminence (ME).[14] Both inducers of CRF release require the presence of calcium ions. CRF has been indicated in the release of other pituitary agents, β-lipotropin and β-endorphin, and it alters the level of excitability of other CNS neurons. These characteristics lead some neuroendocrinologists to speculate that CRF may be a neurotransmitter within the CNS.

Pathophysiology and Clinical Significance

Because it has not yet been specifically characterized, the clinical usefulness of CRF has not yet been elucidated. Once characterized, however, it may lead to methods to

test for secondary and tertiary deficiencies of the hypothalamic-pituitary-adrenal axis. It may also catalyze the development of useful agents to prevent or diminish iatrogenic Addison's disease in those patients who receive long-term steroid therapy. Some cases of Cushing's syndome with adrenal hyperplasia are attributed to increased CRF secretion and therapeutic inhibition or antagonism of CRF secretion is a beneficial treatment.[19]

Thyrotropin Releasing Hormone (TRH)

Identification

The presence of a hypothalamic-releasing hormone for the anterior pituitary thyroid stimulating hormone (TSH) was implied in early studies that related hypothalamic function to thyroid size and activity. Hypothalamic extracts, or electrical stimulation of the hypothalamus, could increase thyroid size and function. Hypothalamic ablation or lesion results in thyroid atrophy and hypofunction.

TRH was described first as thyrotropin releasing factor (TRF), but later was characterized as a tripeptide with guarded N- and C- terminal amino acids (Table 1). TRH was the first hypothalamic hypophysiotropic hormone to be structurally identified and its sequence confirmed by chemical synthesis. Its discovery marked the beginning of our present understanding of hypothalamic regulatory hormones and gave credibility to the then budding, now burgeoning, field of neuroendocrinology.

The discovery of TRH and the procedures for its isolation and purification are presented in detail in the original studies.[20,21] Later reviews are available.[14,15] Generally, porcine or ovine hypothalamic fragments were acid-treated, and purified by gel filtration and ion-exchange chromatography. Final purification procedures involved partition chromatography, adsorption chromatography on charcoal, analytical gel filtration, and terminal purification with paper chromatography.

Biosynthesis, Localization and Degradation

TRH is synthesized *de novo* in specific brain areas by a nonribosomal TRH synthetase that requires both ATP and Mg^{++}.[12] There is some evidence TRH may be a product of proteolytic degradation of some larger moiety (prohormone), as it is distributed widely throughout the CNS where the TRH synthetase has not been found. It has been shown that in vitro biosynthesis occurs in pulsatile bursts, similar to the in vivo secretory pattern observed for TSH.[13]

Radioimmunoassay and immunohistochemical techniques, as well as microfractionation procedures, were developed to localize TRH within the CNS. These procedures and resultant studies are detailed in several excellent recent reviews.[6,9] The highest concentration of TRH is located in the medial portion of the external layer of the median eminence with moderate concentration associated with the pituitary stalk. Nerve terminals containing TRH are located not only within the hypothalamus, but are extrahypothalamic as well (Table 2).[9] Subcellular distribution of hypothalamic peptides indicate that both the hypothalamic and extrahypothalamic TRH are associated with the synaptic vesicles. Furthermore, depletion of hypothalamic TRH can be observed without concomitant change in extrahypothalamic TRH, indicating that TRH biosynthesis may occur outside the hypothalamus. This is one basis for the current premise that TRH may have a neurotransmitter function in many areas of the CNS. TRH has been discovered in all areas of the brain with the exception of the cerebellum and has been isolated in the blood, cerebrospinal fluid (CSF) and urine.

TRH degradation occurs in both the blood and tissues, but not in urine or CSF.[22] Degradation primarily involves enzymatic cleavage of the pyroGlu-His- or His-ProNH_2 bonds, or simple deamination of the peptide.[12] The TRH degrading peptidases are widely distributed within the CNS and peripheral tissues.

Table 2
REGIONS OF THE CNS
CONTAINING THYROTROPIN
RELEASING HORMONE (TRH)

Nerve Terminals Containing TRH
- Hypothalamic areas
  - Dorsomedial nucleus
  - Paraventricular nucleus
  - Perifornical region
  - Ventromedial nucleus
  - Zona incerta
  - Periventricular area
  - Suprachiasmatic nucleus
  - Ventral preoptic area
  - Medial forebrain bundle
  - Organum vasculosum lamina terminalis
- Extrahypothalamic areas
  - Nucleus accumbens
  - Nucleus interstitialis
  - Stria terminalis
  - Lateral septal nucleus
  - Brainstem nuclei (several)
  - Ventral horn
  - Intermediolateral cell column

Cell Bodies Containing TRH
- Lateral hypothalamus
- Dorsomedial nucleus
- Paraventricular nucleus
- Perifornical region
- Periventricular nucleus
- Median eminence

Physiologic Role

TRH is a primary component of the hypothalamic-pituitary-thyroid axis. It stimulates the synthesis and secretion of the large molecular weight glycoprotein moiety, thyroid stimulating hormone (TSH), from the anterior pituitary. Plasma TSH increases within two min following the administration of TRH.[22]

The mechanism by which TRH acts is still unclear, but most likely involves stimulation of membrane-bound adenylate cyclase and the resultant elevation of intracellular cyclic adenosine monophosphate (cAMP).[23] Specific pituitary membrane receptors for TRH have been identified and their affinity appears to be directly influenced by the presence of thyroxine (T_4) and triiodothyronine (T_3).[15] A review of studies characterizing the binding phenomenon and receptor activity was published several years ago[24] and more recently updated.[23] Some recent evidence, utilizing isolated thyroid tumor cells, suggests that there is no alteration of intracellular cAMP level in the presence of TRH.[25]

The TRH stimulating effect upon pituitary secretion of TSH is modulated by the plasma levels of either T_4 or T_3. Tonic levels of TRH may establish a physiologic set-point for TSH secretion. T_4 and T_3 determine the sensitivity of the anterior pituitary to the stimulatory action of TRH.[23] T_4 serves to inhibit the TRH effect upon the adenohypophysis, thus reducing TSH secretion, and it may also inhibit TRH secretion when administered intracranially. A short feedback regulation may also exist for TSH as it may stimulate TRH release from the hypothalamus. This positive feedback regulation is atypical of hormonal control mechanisms and is yet to be elucidated.

TRH stimulation of TSH secretion appears to be opposed by another hypothalamic hormone, somatostatin (SS). Somatostatin is discussed in more detail later in this chapter.

TRH, acting through cAMP, releases not only TSH but prolactin as well. It is uncertain, however, if TRH stimulation of prolactin release occurs within the normal physiologic environment. This stimulatory effect is unexpected, as it is recognized that the primary control of prolactin release is via an inhibitory releasing factor (discussed in more detail later in this chapter).

Normal plasma levels of TRH is approximately 60 pg/mℓ, with primary hyperthyroidism yielding values near 5 pg/mℓ. Plasma levels exceeding 100 pg/mℓ have been noted in pituitary hypothyroidism.[14]

TRH is found in the plasma of man and has a plasma half-life of 4 to 5 min, and a volume distribution of 15.7 liters.[26] TRH causes an increase in the synthesis, secretion, and release of TSH from the pituitary of several species of mammals and birds whereas it fails to affect TSH release in amphibia or fish.

A review of the CNS actions of hypothalamic hormones[8] notes that TRH may act directly upon the brain, stimulating neuronal activity and regulating various aspects of behavior. In general, TRH increases behavioral activity in animals by a mechanism that is independent of the pituitary gland. Intraventricular administration of TRH can enhance the action of several antidepressant drugs and oppose the action of depressants such as pentobarbital and chloral hydrate. TRH appears to be a CNS stimulant. Neurophysiologic studies have identified TRH sensitive neurons, some excitatory and others inhibitory. TRH, like LRH, may be a neurotransmitter. It has been found within vesicles of nerve endings and it is released from nerve terminals. It initiates CNS-dependent behavior and alters neuronal excitability of hypothalamic and extrahypothalamic neurons. These actions support the role of TRH as a neurotransmitter.

Pathophysiology and Clinical Significance

Thyroid dysfunction caused by anomalies of the pituitary gland and/or the hypothalamus may represent only 5 to 10% of the reported cases of thyroid disease. Hypothalamic dysfunction could result from either deficient or excess secretion of TRH. TRH clinically may be utilized as a tool to test for pituitary reserve of TSH, prolactin and, in certain circumstances, growth hormone.[27] Because recent studies show that TRH may be a neurotransmitter and play a role in behavior, TRH and its analogs may be candidates for drug therapy of certain psychic and behavioral diseases. Numerous synthetic analogs have been produced with varying degrees of TSH releasing activity (Table 3).

Luteinizing Hormone Releasing Hormone (LRH)

Identification

Near the turn of the 20th century, clinical observations and surgical techniques showed that the hypothalamus and pituitary are related in some undefined manner to growth of ovarian and testicular structure and development of their function.[28] Studies with crude brain and hypothalamic extracts brought to light two seemingly different factors that independently regulate the release of the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These factors are found in both sexes of humans and other mammals. Originally described as "factors", but now structurally identified, these two agents are generally considered to be a single substance, luteinizing hormone releasing hormone (LRH) (Table 1).

In 1971, the structure of porcine LRH was elucidated by Schally.[29] The decapeptide hypothalamic hypophysiotropic hormone was isolated, purified, characterized, and synthesized within a remarkably short three year span. Since that time, LRH has been

Table 3
ANALOGS OF THYROTROPIN RELEASING HORMONE (TRH)[a]

Structure	TSH releasing activity (TRH = 100)
TRH = Pyr-His-Pro-NH_2	100
D-Pyr-D-His-L-Pro-NH_2	inactive
—His-Pro-NH_2	0.1
Pyr-D-His-Pro-NH_2	2-5
Pyr-His-D-Pro-NH_2	0.1
—Pro-OH	0.02
—OMe	20
—NH-NH_2	14
—NH-Me	20
—NH-Et	14
—$N(Me)_2$	0.5
—$N(Et)_2$	0.05
—NH-CH_2-CH_2-OH	16
—piperidine	0.2
—Gly-NH_2	35
—Ala-NH_2	0.5
Pyr-His-Pyrrolidine	0.2
—prolinol	1.2
—NH_2	0.003
—OMe	inactive
—Gly-NH_2	<0.02
—Ala-NH_2	0.1
—Leu-NH_2	0.04
—Val-NH_2	0.1
—Abu-NH_2	0.1
—Ile-OMe	inactive
—ILe-NH_2	inactive
—Thr-NH_2	inactive
—Met-NH_2	inactive
—Phe-NH_2	inactive
—Trp-NH_2	<0.02
Pyr-Gly-Pro-OMe	inactive
—NH_2	inactive
Pyr-Ala-Pro-NH_2	inactive
Pyr-Leu-Pro-OMe	inactive
—NH_2	0.2
Pyr-Met-Pro-NH_2	1.0
Pyr-N$^{\alpha}$ MeHis-Pro-NH_2	0.04
Pyr-N$^{\tau}$ MeHis-Pro-NH_2	800
Pyr-N$^{\alpha}$ MeHis-OMe	<0.0005
Pyr-N$^{\tau}$ MeHis-OMe	0.02
Pyr-Phe-Pro-OMe	inactive
—NH_2	10
Pyr-Tyr-Pro-NH_2	0.084
Pyr-Trp-Pro-NH_2	inactive
Pyr-Thi-Pro-NH_2	~0.2
Pyr-D-Thi-Pro-NH_2	~0.2
Pyr-Arg-Pro-NH_2	0.05
Pyr-Lys-Pro-NH_2	0.02
Pyr-Orn-Pro-NH_2	0.025
Pyr-His (Bzl)-Pro-NH_2	0.2
Pyr-β-3-pyrazolyl-Ala-Pro-NH_2	5
Acetyl-Glu-His-Pro-NH_2	inactive

Table 3 (continued)
ANALOGS OF THYROTROPIN RELEASING HORMONE (TRH)[a]

Structure	TSH releasing activity (TRH = 100)
H-Glu-His-Pro-OH	inactive
H-Glu-His-Pro-NH_2	5
N-Me-Pyr-His-Pro-NH_2	inactive
H-Pro-His-Pro-OH	inactive
—NH_2	0.01
Thiophenyl-CO-His-Pro-NH_2	0.2
Furanyl-CO-His-Pro-NH_2	0.01
Cyclopentyl-CO-His-Pro-NH_2	<0.01
Cyclobutyl-CO-His-Pro-NH_2	0.016
H-Gly-His-Pro-NH_2	inactive
H-Glu-Pro-His-OH	inactive

[a] See Reference 15.

found to be identical in structure and function in every mammal examined as well as in some birds and fishes. Though the procedures for isolation and purification are reported in detail in the original paper and others,[29,30,31] the general method involves extraction of hypothalamic fragments with either acetic acid or acidified organic solvents, followed by gel filtration with Sephadex G25, phenol extraction, ion exchange chromatography on CM-cellulose, thin layer chromatography, electrophoresis, and terminated with countercurrent distribution techniques. Although early isolation techniques revealed separate fractions for LH and FSH releasing activity, a single entity has been shown to possess both activities. Since LRH was first synthesized, many analogs have been prepared and examined (Table 4).

Biosynthesis, Localization, Degradation and Secretion

Whereas the nonribosomal *de novo* synthesis of tripeptides such as that of TRH is a possible mechanism of biosynthesis, polypeptides the size of LRH and somatostatin are less likely to be produced by this mechanism.[13] Reports of LRH biosynthesis differ and the exact mechanism is still to be proven. LRH biosynthesis occurs in both the particulate and nonparticulate fractions of hypothalamic extracts. However, the strongest evidence suggests that the synthetic mechanisms are associated with the synaptosomal fraction. Additional evidence suggests that the synthesis may result from enzymatic cleavage of a larger molecular precursor (prohormone?). Investigations regarding LRH must be tempered by the difficulty of rigorously proving the identity of the product of these experiments as LRH.

Localization of LRH within the CNS was first determined by bioassay procedures, but more recently antibodies have been prepared against LRH synthesized in the laboratory. This has resulted in the development of radioimmunoassay and histochemical procedures that have localized the distribution of LRH within the CNS and other body compartments.[7,15] LRH is primarily associated with the lateral aspects of the external layer of the median eminence, and it is found associated with the arcuate nucleus. It is also concentrated in the Organum Vasculosum Lamina Terminalis (OVLT) (Table 5). The median eminence is principally devoid of neuronal cell bodies so LRH is associated with axons and nerve terminals.[32] This association of LRH with the OVLT has generated a hypothesis of intraventricular transport of LRH to the median eminence.